PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12358760/TDO/FT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/001356**	International Filing Date *(day/month/year)* 14 October 2003	Priority Date *(day/month/year)* 14 October 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] C12N 15/12 C12N 15/63 C12N 5/16 A61K 48/00 A61K 38/45

Applicant

MEDVET SCIENCE PTY.LTD. et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand 12 May 2004	Date of completion of the report 8 February 2005
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001356

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001356

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be nonobvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos: 20-24, 27, 28, 43 in part.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. 20-24, 27, 28, 43 in part.

These claims are directed to **any agent, analogue, equivalent, mimetic, agonist or antagonist** capable of modulating the functional level of SK. The scope of these terms is such that any and all known and unknown molecules or compounds are encompassed. This is clearly beyond the teaching and scope of the specification. Hence the search was limited to the over-expression of sphingosine kinase and not to any and all molecules or compounds that may modulate SK.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims -	**YES**
	Claims **1 - 43 in part**	**NO**
Inventive step (IS)	Claims -	**YES**
	Claims **1 - 43 in part**	**NO**
Industrial applicability (IA)	Claims **1 - 43 in part**	**YES**
	Claims -	**NO**

2. Citations and explanations (Rule 70.7)

The invention appears to reside in the over-expression of sphingosine kinase in endothelial cells such that the over-expression modulates a functional characteristic of said cell(s).

Claims 1-26 relate to methods of modulating endothelial cell functional characteristics, and/or methods of treatment of conditions caused by aberrant endothelial cell functioning, by inducing over-expression of sphingosine kinase. Said over-expression causes either up or down regulation of the functional characteristic and is achieved through the use of a nucleic acid, protein or functional equivalent, homologue, mimetic, agonist or antagonist of sphingosine kinase.

Claims 27-42 relate to the use of an agent capable of modulating sphingosine kinase in the manufacture of a medicament for the modulation of endothelial cell functional characteristics by inducing over-expression of sphingosine kinase. Said over-expression causes either up or down regulation of the functional characteristic and is achieved through the use of a nucleic acid, protein or functional equivalent, homologue, mimetic, agonist or antagonist of sphingosine kinase.

Claim 43 relates to a pharmaceutical composition comprising a modulatory agent when used in the method of claims 1-26.

The following citations have been considered for the purposes of this opinion:

D1: US 2002042358

D2: WO 2002028906

D3: WO 2002000887

D4: WO 2001085953

D5: WO 2001074837

D6: WO 2000070028

D7: WO 1999012533

D8: WO 1999061581

D9: Katsuma, S. *et al.* 2002. Genes to Cells. 7:1217-1230

D10: Nava, V.E. *et al.* 2002. Experimental Cell Research. 281:115-127.

Continued on Supplemental Sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 20-24, 27, 28, 43 are not fully supported by the description. These claims refer to agents, analogues, equivalents, mimetics, agonists and antagonists capable of modulating the functional level of sphingosine kinase. Such terms encompass any and all known and unknown molecules. In contrast the description provides for the over-expression of sphingosine kinase by introducing more copies into the cell. No other methods of modulating sphingosine kinase are disclosed. Therefore the description does not provide support for the full scope of these claims.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

D11: Ancellin, N. *et al.* 2002. The Journal of Biological Chemistry. 277(8):6667-6675.

D12: Shu, X. *et al.* 2002. Molecular and Cellular Biology. 22(22):7758-7768.

D13: Vann, L.R. *et al.* 2002. The Journal of Biological Chemistry. 277(15):12649-12656.

D14: Spiegel, S. *et al.* 2002. The Journal of Biological Chemistry. 277(29):25851-25854.

D15: Alemany, R. *et al.* 2001. FEBS Letters. 509:239-244.

NOVELTY(N) and INVENTIVE STEP(IS)

D1 discloses the inhibition or stimulation of sphingosine kinase activity to modulate functional characteristics of cells eg enhancement of cell proliferation. HEK293 (embryonic kidney cells), Swiss 3T3 and NIH 3T3 fibroblasts were transfected with human sphingosine kinase resulting in marked increases in sphingosine kinase activity with resultant changes in sphingolipid metabolites. Further disclosed is that cells over-expressing sphingosine kinase are useful in the study of intracellular actions of sphingosine-1-phosphate (S1P). Also disclosed are methods of testing agents effecting sphingosine kinase activity.

Claims 1-43 lack an inventive step in light of D1.

The only apparent difference between the claimed invention and D1 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase. Furthermore it is well known that sphingosine-1-phosphate regulates diverse biological processes and that sphingosine kinase is responsible for the formation of S1P. Hence there is not considered to be any inventive merit in over-expressing a known protein which produces a second protein that effects a diverse range of biological processes. Any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D1 as D1 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

D2 discloses the transfection of HEK293 cells with constructs containing SK (examples) as well as methods of identifying modulators of SK activity ie inhibitors or enhancers (p40-49) and their use as pharmaceutical compositions (p49-63) for therapeutic use eg in treatment of inflammatory diseases such as asthma as well as cardiovascular diseases (p52-60).

Thus claims 1-43 lack an inventive step in light of D2.

The only apparent difference between the claimed invention and D2 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase. Further it is well known that sphingosine-1-phosphate regulates diverse biological processes and that sphingosine kinase is responsible for the formation of S1P. Hence there is not considered to be any inventive merit in over-expressing a known protein which produces a second protein that effects a diverse range of biological processes.

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Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

Thus claims directed to the modulation of cellular functional characteristics by over-expressing SK are not considered to involve an inventive step in light of the disclosure of D2. Furthermore, any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D2 as D2 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

D3 discloses the transfection of HEK293 cells with an SK mutant having a glycine to aspartic acid substitution at position 82 such that the introduced SK is catalytically inactive. Hence the introduced gene suppresses increases in activity of SK after treatment of cells with activating agents. Hence D3 discloses methods of modulating cells by over-expressing SK in an effort to modulate the functional characteristics of the transfected cells. Further disclosed is the use of the SK variant in methods of treatment and as a pharmaceutical composition.

Claims 1-43 lack an inventive step in light of D3.

The only apparent difference between the claimed invention and D3 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase in order to modulate its activity and hence the activity of functional characteristics of the transfected cells. Furthermore, any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D3 as D3 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

D4 discloses the transfection of NIH 3T3 fibroblasts with human SK with subsequent increase in SK activity and S1P formation. Stable expression of SK dramatically enhanced cell growth and proliferation. Also disclosed is the use of agents, both proteinaceous and non-proteinaceous to modulate the activity of SK as well as the use of such agents in methods of treatment and pharmaceutical compositions to either up-regulate or down-regulate functional characteristics of transfected cells.

Claims 1-43 lack an inventive step in light of D4.

The only apparent difference between the claimed invention and D3 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase in order to modulate its activity and hence the activity of functional characteristics of the transfected cells. Further it is well known that sphingosine-1-phosphate regulates diverse biological processes and that sphingosine kinase is responsible for the formation of S1P. Hence there is not considered to be any inventive merit in over-expressing a known protein which produces a second protein that effects a diverse range of biological processes. Thus claims directed to the modulation of cellular functional characteristics by over-expressing SK are not considered to involve an inventive step in light of the disclosure of D4. Furthermore, any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D4 as D4 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

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(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

D5 discloses the transfection of HEK293 and NIH 3T3 fibroblasts with vectors containing SK constructs and subsequent measurements of the increased activity of SK and S1P. Further a number of inhibitors and enhancers of SK activity were also analysed as well as methods of treatment and pharmaceutical compositions comprising modulators of SK activity.

Claims 1-43 lack an inventive step in light of D5

The only apparent difference between the claimed invention and D5 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase in order to modulate its activity and hence the activity of functional characteristics of the transfected cells. Further it is well known that sphingosine-1-phosphate regulates diverse biological processes and that sphingosine kinase is responsible for the formation of S1P. Hence there is not considered to be any inventive merit in over-expressing a known protein which produces a second protein that effects a diverse range of biological processes. Thus claims directed to the modulation of cellular functional characteristics by over-expressing SK are not considered to involve an inventive step in light of the disclosure of D5. Furthermore, any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D5 as D5 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

D6 discloses the isolation and characterisation of SK from HUVEC cells as well as the use of TNFα to stimulate SK expression and activity (examples). HEK293 cells are also transfected with SK. Further disclosed is that SK is the key element in the SK signalling pathway which is known to regulate cellular activities which lead to inflammation, apoptosis and cell proliferation such that modulation of expression of SK modulates one or more specific functional activities of a cell. The citation also discloses therapeutic and prophylactic uses of SK as well as agonists and antagonists of SK for the regulation of cellular functional activity (p.28-41)

Claims 1-43 lack novelty and/or an inventive step in light of D6.

D6 appears to disclose all the essential features of the claimed invention. Hence claims 1-43 lack novelty in light of D6.

Furthermore it is not clear where the applicant's advance over the disclosure of D6 lies, hence the claimed invention also lacks an inventive step in light of D6.

D7 discloses the modulation of cellular characteristics of HUVECs by modulating the activity of SK either by up-regulation or down-regulation using agents, agonists and antagonists. The use of such agents as pharmaceuticals and in methods of treatment to modulate endothelial cell adhesion molecule expression and diseases such as coronary heart disease.

Thus as all the essential features of the claimed invention are disclosed by D7, claims 1-43 lack novelty.

Furthermore it is not clear where the applicant's advance over the disclosure of D7 lies, hence the claimed invention also lacks an inventive step in light of D7.

Continued on Supplemental Sheet

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

D8 discloses the cloning and characterisation of SK as well as its transfection into HEK293, NIH 3T3 and Swiss 3T3 with subsequent increase in SK activity. Further disclosed is the effect on S1P, cell proliferation, apoptosis and chemotaxis of over-expression of SK (examples). Furthermore D8 discloses agents acting as inhibitors and enhancers of SK activity and the potential for such agents in methods of treatment and use as pharmaceuticals.

Claims 1-43 lack an inventive step in light of D8.

The only apparent difference between the claimed invention and D5 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase in order to modulate its activity and hence the activity of functional characteristics of the transfected cells. Further it is well known that sphingosine-1-phosphate regulates diverse biological processes and that sphingosine kinase is responsible for the formation of S1P. Hence there is not considered to be any inventive merit in over-expressing a known protein which produces a second protein that effects a diverse range of biological processes. Thus claims directed to the modulation of cellular functional characteristics by over-expressing SK are not considered to involve an inventive step in light of the disclosure of D8. Furthermore, any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D8 as D8 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

D9 discloses the transfection of SK into mesangial cells with subsequent measurement of SK and S1P activity and levels. Further disclosed is the importance of SK and S1P in the modulation of cellular processes such as cell proliferation. Inhibitors and enhancers of SK expression are also disclosed.

Claims 1, 2 and their dependent claims lack an inventive step in light of D9.

The only apparent difference between the claimed invention and D9 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase in order to modulate its activity and hence the activity of functional characteristics of the transfected cells. Further it is well known that sphingosine-1-phosphate regulates diverse biological processes and that sphingosine kinase is responsible for the formation of S1P. Hence there is not considered to be any inventive merit in over-expressing a known protein which produces a second protein that effects a diverse range of biological processes. Thus claims directed to the modulation of cellular functional characteristics by over-expressing SK are not considered to involve an inventive step in light of the disclosure of D9. Furthermore, any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D9 as D9 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

Continued on Supplemental Sheet

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

D10 discloses that SK and S1P are implicated in cell growth, transformation and cancer. Also disclosed is that over-expression of SK in MCF-7 cells (breast cancer) enhances proliferation and reduces apoptosis.

Claims 1, 2, 3-26 lack an inventive step in light of D10.

The only apparent difference between the claimed invention and D10 is the different cell types used. There is not considered to be any inventive merit in selecting different cell types in which to over-express sphingosine kinase in order to modulate its activity and hence the activity of functional characteristics of the transfected cells. Further it is well known that sphingosine-1-phosphate regulates diverse biological processes and that sphingosine kinase is responsible for the formation of S1P. Hence there is not considered to be any inventive merit in over-expressing a known protein which produces a second protein that effects a diverse range of biological processes. Thus claims directed to the modulation of cellular functional characteristics by over-expressing SK are not considered to involve an inventive step in light of the disclosure of D10. Furthermore, any differences between the claimed invention and that of the citation are regarded as technical equivalents and do not add to the inventiveness of the claimed invention.

Claims 1-43 are considered novel in light of D10 as D10 doesn't disclose a method of modulating endothelial cell functional characteristics by over-expressing sphingosine kinase.

D11 discloses that over-expression of SK resulted in enhanced release of SK activity, S1P formation and induced angiogenesis and vascular maturation. Inhibitory and enhancer agents were also analysed in relation to these processes.

Hence claims 1, 2, 4-26 lack novelty and/or an inventive step. It would appear that all the essential features of at least the independent claims are disclosed by D11. Additionally, any differences between the claimed invention and that of the citation are regarded as technical equivalents and are not considered to add to the inventive merit of the invention. Hence the claimed invention also lacks an inventive step.

D12 discloses that VEGF stimulates sphingosine kinase which effects endothelial cell signalling. This citation would be relevant to the novelty and inventiveness of all claims were the priority of the present application found to be invalid. As the priority of the present application appears valid, D12 is not relevant to the novelty or inventiveness of the claimed invention.

Claims 1-43 are considered novel and inventive in light of D13-D15 as these citations don't disclose or suggest the claimed invention.

INDUSTRIAL APPLICABILITY(IA)

Claims 1-43 meet the requirements under the PCT in respect of industrial applicability.